Current Biology, Vol. 14, R674–R685, August 24, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.08.017

Review

Dare to Be Different: Asymmetric Cell Division in *Drosophila*, *C. elegans* and Vertebrates

Jörg Betschinger[1] and Jürgen A. Knoblich[2]

One widespread mechanism for the generation of diverse cell types is the unequal inheritance of cell fate determinants. Several such determinants have been identified in the fruitfly *Drosophila melanogaster* and the worm *Caenorhabditis elegans* and the molecular machinery responsible for their asymmetric segregation is beginning to be unraveled. To divide asymmetrically, cells establish an axis of polarity, orient the mitotic spindle along this axis and localize cell fate determinants to one side of the cell. During cytokinesis, determinants are then segregated into one of the two daughter cells where they direct cell fate. Here, we outline the steps and factors that are involved in this process in *Drosophila* and *C. elegans* and discuss their potential conservation in vertebrates.

Introduction

Every organism consists of a variety of different cell types. To generate this diversity, cells can form two different cell types from one. This can be achieved in two ways [1]: either, two initially identical daughter cells become different because they encounter different environments; or, alternatively, cell fate determinants are segregated into only one of the two daughter cells during mitosis to make this cell different from its sister cell (Figure 1A). Although such intrinsically asymmetric cell divisions had already been postulated in 1905 [2] (Figure 1B,C), the first segregating determinant was molecularly characterized only 90 years later [3]. Today, the significance of asymmetric cell divisions for the development of multicellular organisms, including humans, is widely recognized. Of particular importance is the asymmetric nature of stem cell divisions: Stem cells must generate daughter cells that are committed to differentiation, while others maintain stem cell characteristics. Accumulating evidence suggests that intrinsically asymmetric cell divisions are involved in making this distinction, particularly in the vertebrate nervous system. Most of our mechanistic insight into this process comes, however, from invertebrate model systems, especially *Drosophila* and *C. elegans*.

[1]Research Institute of Molecular Pathology (I.M.P.), Dr. Bohr Gasse 7, 1030 Vienna, Austria. E-mail: betschinger@imp.univie.ac.at [2]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Dr Bohr Gasse 3-5, 1030 Vienna, Austria. E-mail: knoblich@imp.univie.ac.at

Model Systems for Asymmetric Cell Division

Both *C. elegans* and *Drosophila* development rely heavily on asymmetric cell divisions [4,5]. In *C. elegans*, early development is essentially a series of asymmetric cell divisions, and especially the first division of the zygote has been intensely studied [6–8]. In *Drosophila,* asymmetric divisions have been described in developing muscle, gut, malphighian tubules and nervous system, and in particular the asymmetric divisions of neuroblasts in the central nervous system and of sensory organ precursors in the peripheral nervous system are well studied [9,10].

In *C. elegans* (Figure 2A), polarization starts with sperm entry into the oocyte, whose position defines the posterior end of the zygote. The zygote — also called P0-cell — divides asymmetrically along the anterior–posterior axis (note that we will use the orientation of the mitotic spindle to define the orientation of the cell division) and produces a larger, anterior AB-cell and a smaller posterior P1-cell . The two daughter cells are different in size and committed to distinct fates: The AB-cell will mainly form ectoderm, whereas P1 will give rise to the germline, as well as endo- and mesoderm.

In the *Drosophila* central nervous system (Figure 2B), progenitor cells, called ‘neuroblasts’ are specified within a monolayered epithelium, the ventral neuroectoderm. They delaminate from the epithelium, come to lie beneath the epithelial cell layer and undergo repeated rounds of asymmetric cell division in a stem cell like fashion. Each division gives rise to a small basal daughter cell called ganglion mother cell (GMC) and a larger apical daughter cell. The GMC divides only once more to form neuron and glia cells, whereas the apical daughter cell continues to divide asymmetrically.

In the *Drosophila* peripheral nervous system (Figure 2C), sensory organ precursor (SOP) cells give rise to the four cells that make up the external sensory organs. SOP-cells, also known as pI-cells, are specified in the epithelium and divide asymmetrically within the plane of the epithelium, along the anterior–posterior axis, to generate an anterior pIIb and a posterior pIIa cell. pIIa and pIIb are different in morphology and developmental potential: Both continue to divide asymmetrically and the pIIb-descendents form the internal structures of the sensory organ, whereas the pIIa generates its external structures. pIIb cells also give rise to a glia cell that will undergo apoptosis and not become part of the organ.

Despite different developmental requirements and potential in these three systems, asymmetric cell divisions can be seen as proceeding through four steps (Figure 1A): First, before division, an axis of polarity is established that is coordinated with the body axes. Second, the mitotic spindle is set up and oriented along the axis of polarity. Third, cell fate determinants

are distributed in a polarized fashion along this axis. This ensures their unequal inheritance by only one of the two daughter cells after cell division. Fourth, different concentrations of these determinants in the two daughter cells lead to the establishment of distinct cell fates. Below, we will discuss these four steps in *C. elegans* and *Drosophila* and describe the molecules and mechanisms involved.

Setting Up Polarity

The key players for setting up polarity were identified in a pioneering genetic screen by Ken Kemphues and colleagues (Figure 3A) [11]. This screen for mutants affecting the first asymmetric cell division of the *C. elegans* zygote identified the so-called *par* genes (partitioning-defective) *par-1* to *par-6*. *pkc-3*, a seventh member of this group, was identified later by homology to an atypical protein kinase C (aPKC) [12] . In *par* mutants, size and fate differences between the two daughter cells, P1 and AB, are less pronounced, and in extreme cases the two cells are identical. With the exception of Par-2, all Par-proteins are conserved during evolution [13,14]. Their homologs regulate epithelial polarity in *Drosophila* and vertebrates, and are involved in cell migration and the establishment of the *Drosophila* anterior–posterior body axis [13].

Despite their functional similarity, Par-proteins are quite divergent in sequence: Par-1, Par-4 and aPKC are Ser/Thr-kinases, Par-3 (called Bazooka in *Drosophila*) and Par-6 are PDZ domain proteins, Par-2 contains a RING-finger and Par-5 is a member of the 14-3-3 class of proteins.

According to their localization in *C.elegans*, Par-proteins can be grouped into three classes [8]: Par-3, Par-6 and aPKC localize to the anterior cell cortex, whereas Par-1 and Par-2 are found at the posterior cortex. Par-4 and Par-5, by contrast, are distributed uniformly at the cell cortex. Of these three classes, only Par-3, Par-6 and aPKC have an evolutionarily conserved role in asymmetric cell division [14]. The three proteins bind to each other and form the Par-3/6 complex, which localizes apically in *Drosophila* neuroblasts and anteriorly in SOP-cells [9]. The asymmetric localization of this complex reflects the establishment of an axis of polarity which is essential for spindle orientation and asymmetric protein localization during mitosis. In *Drosophila* neuroblasts, inherited epithelial apical-basal polarity is thought to establish Par-protein localization, whereas in SOP-cells epithelial planar polarity cues are responsible for Par-protein distribution. In all three systems, Par-protein localization proceeds through distinct establishment and maintenance phases (Figure 3B).

In *C. elegans*, the point of sperm entry defines where the Par-proteins will accumulate. After fertilization, the area of the cell cortex that overlies the sperm derived centrosome is depleted of Par-3/6, thus allowing for the posterior accumulation of Par-2 [15,16]. Recent experiments suggest that the interaction of the centrosome with the cell cortex is independent of microtubules [17]. Instead, the redistribution of the Par-proteins involves a directional and actin-myosin dependent flow of the cortical cytoplasm [18]. This

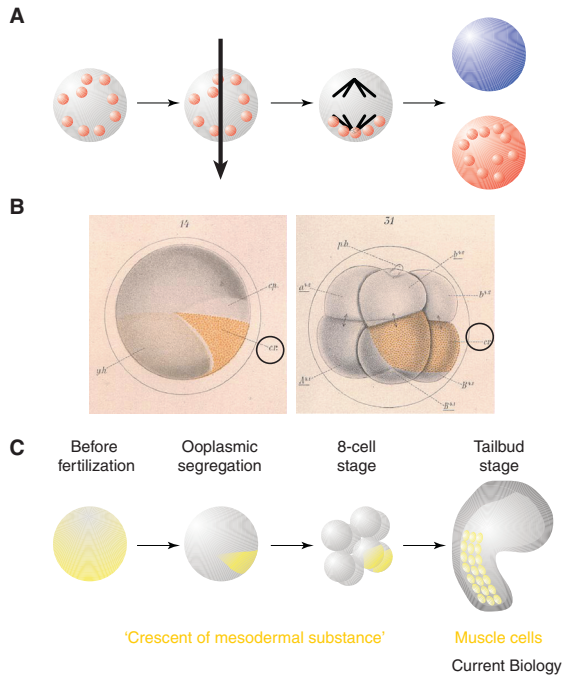

Figure 1. Asymmetric cell divisions.
(A) During asymmetric cell divisions, progenitor cells polarize along an axis of polarity (arrow) that directs the orientation of the mitotic spindle and the asymmetric localization and segregation of cell fate determinants (red balls). (B) In ascidian embryos, yellow pigment (*cr* = crescent of mesodermal substance; marked by circles) segregates into muscle cells of the tadpole. The figure shows Ed Conklin's original drawings of a one-cell stage (left) and eight cell stage (right) embryo [2]. (C) Schematic representation of Conklin's observations highlighting the asymmetric localization and segregation of yellow pigment.

cortical flow is essential for the establishment of Par-protein localization, but also requires most of the Par-proteins; this indicates a mutual interaction between Par-proteins and the cortical actin cytoskeleton. Possibly, Par-proteins propagate an initial weak cortical flow that is initiated by the sperm centrosome. Anterior Par-3/6 localization becomes dependent on posterior Par-2 when the sperm centrosome duplicates and cortical flow ceases [16]. Similarly, the small GTPase Cdc42 — a binding partner of Par-6 [19] – is only required for maintenance but not for establishment of Par-protein localization [19]. Thus, polarity in the *C. elegans* zygote is established by interactions between the centrosome and the cell cortex that induce a cortical flow and polarize Par-protein distribution. After the cortical flow has ceased, the domains are maintained by mutual exclusion of anterior and posterior Par-proteins.

Polarization of *Drosophila* neuroblasts proceeds through similar establishment and maintenance phases. Polarity is established when neuroblasts become specified in the polarized epithelium of the neuroectoderm where Par-3/6 are concentrated in the so-called 'subapical region', which is located just apical to the adherens junctions [20]. When neuroblasts delaminate from the epithelium, Par-3/6 are found in a stalk that extends into the epithelial layer and, after

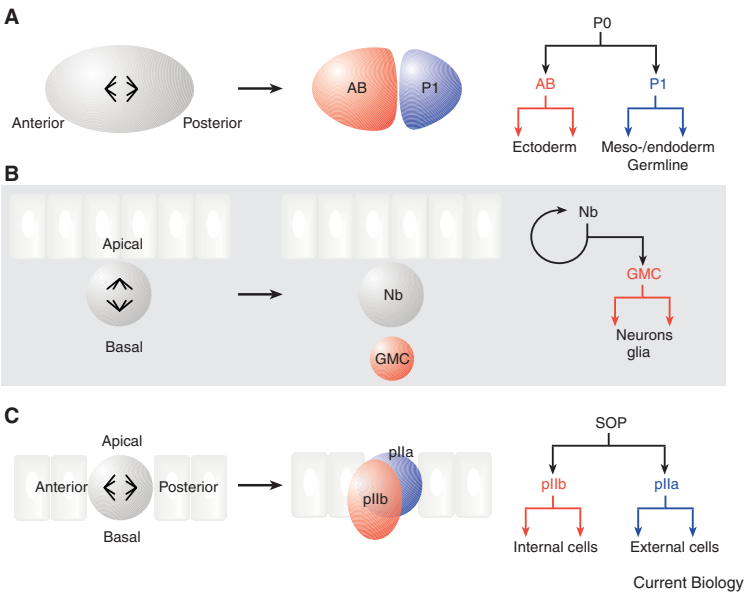

Figure 2. Model systems for asymmetric cell division.
(A) The *C. elegans* zygote divides along its anterior–posterior axis and gives rise to a bigger anterior AB and a smaller posterior P1 daughter cell, which are committed to different cell lineages. (B) *Drosophila* neuroblasts are located underneath the neuroectoderm. They divide along their apical–basal axis and generate a bigger apical daughter cell that retains neuroblast characteristics. The smaller basal ganglion mother cell (GMC) divides once more into neuronal and glial cells. (C) *Drosophila* sensory organ precursor (SOP) cells divide in an epithelial cell layer along their anterior–posterior axis and generate two nearly equal sized morphologically different daughter cells that form the internal and external cells of the sense organ.

delamination, they occupy the apical cell cortex [20–24]. This has led to the hypothesis that neuroblasts inherit their apical-basal polarity from the overlying epithelium [21,22]. After neuroblast delamination, an adaptor protein called Inscuteable starts to be expressed [25]. Inscuteable binds to the Par-3/6 complex and colocalizes with the complex in the stalk during delamination as well as on the apical cell cortex in delaminated neuroblasts [21,22]. Inscuteable, in turn, recruits another adaptor protein, Pins (Partner of Inscuteable, also called Rapsynoid), and the heterotrimeric G protein α-subunit Gαi into the complex [26–29]. Localization of Inscuteable, Pins and Gαi requires the Par-3/6 complex at all stages [21,22]. Localization of the Par-3/6 complex itself, however, passes through two distinct stages: In the stalk, Par-3/6 localization is independent of Inscuteable/Pins/Gαi. However, when neuroblasts are fully delaminated the localization of all proteins becomes partially co-dependent [21,22,27,29,30]. In the absence of one member, the others become partially delocalized to various degrees, which indicates a partial redundancy of the individual components [30]. These two stages coincide with the neuroblasts losing contact to neighboring cells and are likely to reflect a transition from a contact mediated polarization in epithelial cells to cell autonomous polarity in neuroblasts. Consistent with this, asymmetric protein localization can be maintained in individual, cultured neuroblasts, whereas epithelial cell polarity depends on adhesion to neighbors or to the substratum [31]. How G-protein signaling can maintain cell-polarity is completely unclear. In yeast, an autoregulatory loop involving heterotrimeric G-proteins and Cdc42 maintains polarity, but evidence for a similar loop in neuroblasts is still missing [32].

Like neuroblasts, *Drosophila* SOP-cells are specified in an epithelium. For asymmetric division, however, they ignore epithelial apical-basal polarity and, instead, orient along an axis of planar cell polarity [33–36]. Planar cell polarity defines an axis within the plane of the epithelium, which is evident from the unequal subcellular distribution of certain proteins along this axis and the polarized differentiation of cells, for example polarized outgrowth of epidermal hairs [37]. In *Drosophila*, the serpentine receptor Frizzled (Fz) and the transmembrane protein Strabismus (Stbm) are at the heart of a genetic cascade for planar cell polarity. SOP-cells arise from planar-polarized epithelial cells. They inherit the posterior localization of Fz and anterior localization of Stbm from the epithelium and, therefore, planar polarity establishes two opposite cortical domains even before mitosis [36].

As in neuroblasts, both Par-3/6 and Pins/Gαi are important for translating polarity into asymmetric cell division, although the way they act is characteristically different: Stbm binds to Pins and recruits Pins to the anterior cortex during prophase; together, both proteins restrict Par-3/6 to the opposite, posterior side of the cell [36]. Unlike in neuroblasts, Inscuteable is not expressed in SOP-cells and this is why in SOP-cells Par-3/6 and Pins/Gαi localize to opposite sides of the cortex. Upon ectopic expression of Inscuteable, the Par-3/6 complex localizes to the anterior cell cortex, which is occupied by Pins, resulting in an inversion of polarity in SOP-cells [35]. Pins also binds to a MAGUK (membrane associated guanylate kinase) protein called Discs large (Dlg). Pins induces the anterior localization of Dlg and both are required to maintain cell polarity in SOP-cells [35]. Thus, polarization of SOP-cells proceeds through an establishment phase in which planar cell polarity is used to polarize Pins distribution and a maintenance phase where Pins/Gαi and Par-3/6 localize to opposite sides but planar cell polarity is no longer required [35].

Spindle Positioning

Mitotic spindles are important for the generation of asymmetry during mitosis in two ways: First, the orientation of the mitotic spindle needs to be coordinated with the asymmetric localization of cell fate

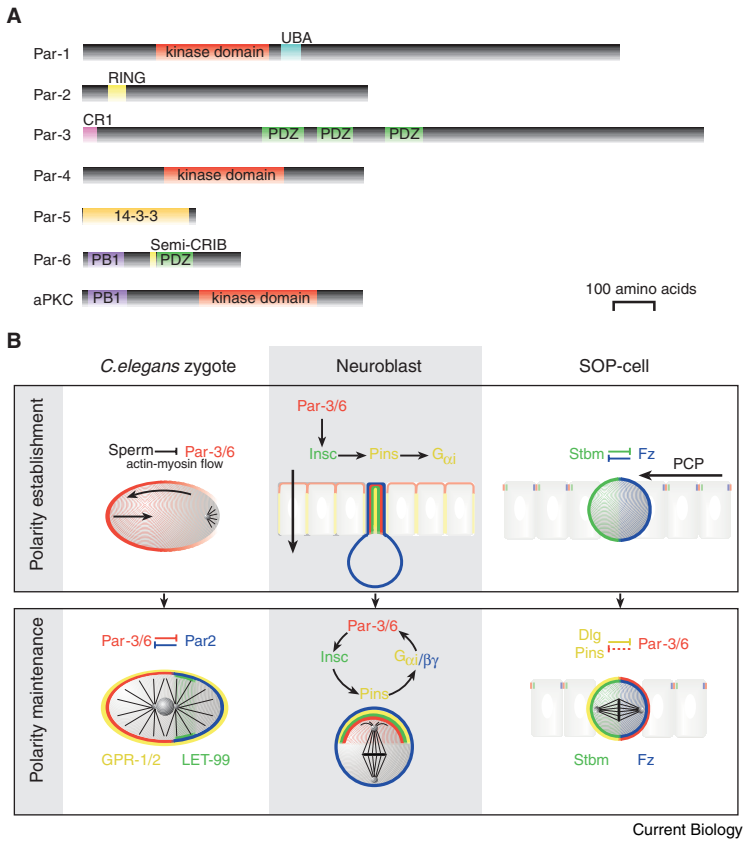

Figure 3. Par proteins set up polarity in asymmetrically dividing cells.
(A) Domain structure of Par-proteins. 14-3-3 binds to phosphoserines and -threonines. CR1 (conserved region 1) is required for Par3-oligomerization. CRIB (Cdc42/Rac-interactive binding) binds GTP-bound Rac/Cdc42 family members. PB1 (Phagocyte oxidase/Bem1) binds other PB1 domains. PDZ (PSD-95, Discs large, Zona occludens-1) binds other PDZ domains and certain carboxy-terminal motifs. RING is mostly associated with E3 ubiquitin-protein ligase activity. UBA (ubiquitin associated domain) is found in several proteins connected to ubiquitin pathways. (B) Polarity establishment and maintenance phases during asymmetric cell division. In the *C. elegans* zygote (left), polarity is initiated by sperm entry inducing an actin-myosin dependent cytoplasmic flow that displaces Par-3/6 from the cortex and allows Par-2 to localize to the posterior cell cortex. When flow ceases, cell polarity becomes dependent upon mutual inhibition between Par-3/6 and Par-2. In *Drosophila* neuroblasts (middle), apical-basal polarity is inherited from the polarized neuroectoderm (arrow) during delamination when Par-3/6 localize in the neuroblast stalk and recruit Insc, Pins and Gαi. When the neuroblast comes to reside beneath the epithelial cell layer, Insc, Pins and heterotrimeric G-proteins become necessary for the maintenance of polarity. In SOP-cells (right), anterior–posterior polarity is initiated by the planar cell polarity (PCP) proteins Fz and Stbm. Stbm recruits Pins to the anterior cell cortex. Together the two proteins restrict Par-3/6 to the opposite cell cortex. Maintenance of polarity during mitosis requires inhibition of Par-3/6 by Pins and its binding partner Dlg but probably a reciprocal inhibition by Par-3/6 as well.

determinates to ensure their asymmetric inheritance. Second, an asymmetric position or shape of the mitotic spindle can create daughter cells of different sizes. Elegant experiments carried out mainly in *C. elegans* have demonstrated that orientation and asymmetry of the mitotic spindle depend on microtubules that emanate from the spindle poles and contact the cell cortex [38–41]. During asymmetric cell division, cortical polarity set up by the Par-proteins modulates interactions between microtubules and the cortex, so that different pulling forces act on the two centrosomes of the mitotic spindle. In *C. elegans*, this leads to a displacement of the spindle toward the posterior end, whereas in *Drosophila* SOP-cells the posterior centrosome is pushed off the cell cortex [34]; in neuroblasts, the mitotic spindle itself becomes asymmetric [42]. In all cases, the daughter emerging from the cell half containing Par-3/6 is bigger than its sister. The differences in spindle asymmetry between the model systems might either be due to different, unrelated mechanisms for generation of spindle asymmetry; alternatively, they could be due to similar mechanisms but different outputs of Par-dependent astral microtubule and centrosome regulation. This latter possibility is supported by the fact that heterotrimeric G-proteins play an important — but highly unexpected — role in spindle orientation and displacement in *Drosophila* and *C. elegans*.

Heterotrimeric G-proteins consist of α-, β- and γ-subunits and roles in spindle orientation have been shown for all three of them. In *C. elegans*, inactivation of the β-subunit GPB-1 leads to defects in centrosome separation as well as spindle orientation and dynamics [43,44]. Similar defects are observed upon depletion of the associated γ-subunit GPC-2 [45]. When the two redundantly acting Gα-subunits GOA-1 and GPA-16 are simultaneously inactivated, the mitotic spindle of the first division fails to displace posteriorly and two identically sized daughter cells are formed [45]. In *Drosophila* neuroblasts mutant for the GPB-1 homolog Gβ13F, mitotic spindles are not correctly oriented [29], and comparable defects are observed in *Gγ1* or *Gαi* mutants [46,47]. Despite this apparent similarity, there are characteristic differences between *Drosophila* and *C. elegans* G-proteins. First, polarity and asymmetric Par-protein localization do not require G-proteins in *C. elegans* [45]. In *Drosophila* G-protein mutants, however, apical localization of Inscuteable and Pins is lost and apical localization of Par-3/6 is strongly reduced [29,46,47]. Second, heterotrimeric G-proteins are not asymmetrically localized in *C. elegans* [48], wheras the α-subunit Gαi colocalizes with the Par-3/6 complex at the apical cell cortex in neuroblasts and localizes asymmetrically to the cortical area opposite to the Par-3/6 complex in SOP-cells [29]. Third, *C. elegans* Gα-proteins regulate Gβ-localization

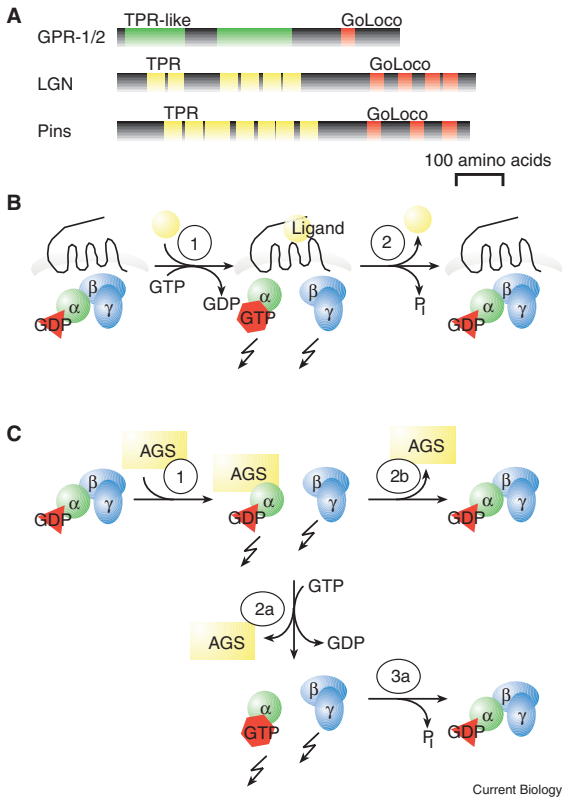

Figure 4. Two modes of heterotrimeric G protein signaling.
(A) Domain structure of AGS (activator of G-protein signaling) proteins. TPR (Tetratrico-peptide repeat) is involved in protein interactions. GoLoco (Gαi/o–Loco interaction motif) binds to GDP-bound heterotrimeric Gαi/o-subunits. (B) Ligand-bound G-protein coupled receptors act as guanine exchange factors (GEF) forming GTP-bound Gα, thereby dissociating and activating α- and β/γ-subunits (1). Signaling is terminated by the intrinsic GTPase activity in Gα-subunits and the reformation of the inactive heterotrimer (2). (C) AGS proteins dissociate the heterotrimeric complex by binding to GDP-bound Gαi/o subunits (1). The AGS-Gα complex can be converted by GEF proteins into GTP-bound Gα (2a). Signaling is terminated either by release of AGS proteins from the AGS-Gα complex (2b) or by the GTPase activity of Gα (3a).

[45], while Gβ/γ control Gαi protein stability in *Drosophila* neuroblasts [29,46].

Heterotrimeric G-proteins are well known for their role in transducing extracellular signals via seven transmembrane (or serpentine) receptors [49] (Figure 4B). Upon ligand binding to the receptor, GDP is exchanged for GTP on the α-subunit. This leads to dissociation of the β/γ- from the α-subunit and of all three subunits from the receptor. Signaling is terminated by the GTPase activity of the α-subunit. Several results indicate that the role of G-proteins during asymmetric cell division is independent of extracellular signals: The *C. elegans* zygote is surrounded by an impermeable membrane and is unlikely to be polarized by extracellular signals. *Drosophila* neuroblasts divide asymmetrically even when they are separated from their environment and cultured as single cells [31]. Instead of extracellular signals, proteins containing so-called GoLoco domains seem to mediate activation of G-proteins [50] (Figure 4). GoLoco domains can bind to α-subunits and trigger the dissociation of β/γ-subunits without the need for receptor activation or GDP/GTP exchange [51].

In *Drosophila*, the GoLoco domain protein Pins (Figure 4A) was identified as a binding partner of Inscuteable [26,27]. In neuroblasts, Pins colocalizes with Gαi on the apical cell cortex and, as *pins* mutants show defects similar to those observed in *Gαi* mutants, Pins seems to be essential for Gαi-activity during asymmetric cell division [26,27,47].

In *C. elegans*, the closest homolog of *pins* is not involved in asymmetric cell division [29]. Instead, two other GoLoco domain proteins called GPR-1 and GPR-2 (Figure 4A) have been identified in an RNAi screen for defects in cell division [52]. GPR-1 and GPR-2 are almost identical and inactivation of both genes causes defects very similar to GOA-1/GPA-1 mutants, indicating that these proteins are functional homologs of Pins in *C. elegans* [44,53–55]. Unlike their *Drosophila* counterparts, GPR-1/2 do not colocalize with Par-3/6. Instead, they are concentrated at the opposite, posterior pole during anaphase where they are thought to increase G-protein activity to polarize the mitotic spindle. GPR-1/2 are counteracted by Let-99, a non-conserved DEP domain protein, that is concentrated in the medial area of the cortex and restricts G-protein activity to the poles [44,56]. Let-99 and GPR-1/2 localization require Par-3/6, indicating that a linear genetic hierarchy is responsible for spindle displacement in *C. elegans*.

In *Drosophila* neuroblasts, a similar genetic hierarchy does not exist. *par-3/6*, *Gαi*, *Gβ13F* or *pins* mutants only have partially penetrant defects in spindle asymmetry and neuroblasts only form equally sized daughter cells when two of these complexes are inactivated simultaneously [30,47,57]. Unlike in *C. elegans*, therefore, Par-3/6 seem to have functions other than polarizing the activity of Gαi. Involvement of other G-proteins (like Gαo) might be an explanation. Alternatively, Par-3/6 could directly act on the mitotic spindle. Such a role is supported by the recent discovery that mammalian Par-3 can bind a microtubule plus-end directed kinesin motor [58].

How do G-proteins act on the mitotic spindle? Laser cutting experiments have shown that pulling forces acting on the astral microtubules are higher in the posterior than in the anterior half of the *C. elegans* zygote [41]. When centrosomes are disintegrated by high intensity laser light, fragments of the remaining microtubule asters are pulled to the cell cortex. The speed of their cortical movement demonstrates that larger net pulling forces arise from a higher number of force generators present at the posterior cell cortex [59]. Upon inactivation of G-proteins or GPR-1/2, pulling forces are mostly lost from the entire cortex, indicating that G-proteins act everywhere but their activity is enhanced posteriorly [53,59]. Thus, heterotrimeric G-proteins are required either to connect microtubules to the cell cortex or to activate molecular motors which pull on these microtubules. Spindle orientation in *C. elegans* requires the dynein/dynactin complex, a microtubule minus end directed motor

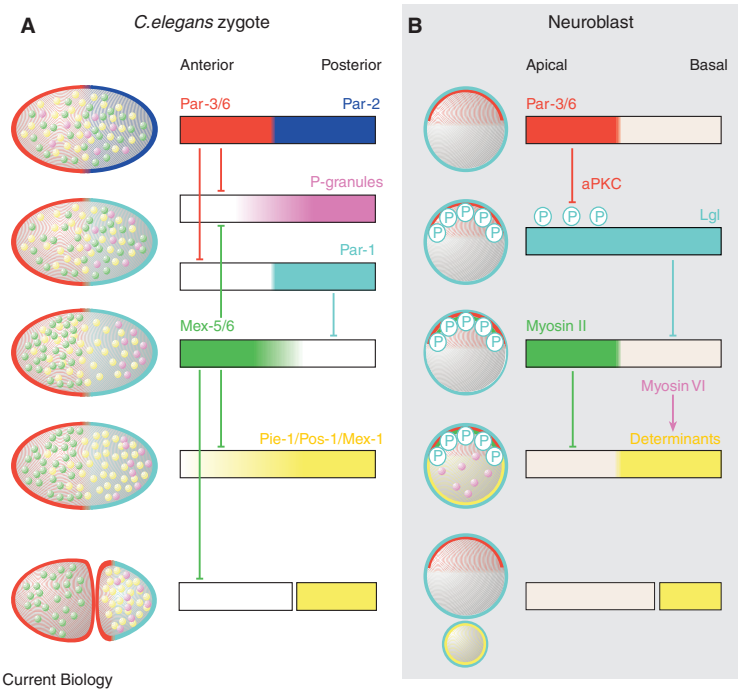

Figure 5. Segregation of cell fate determinants during asymmetric cell division.
(A) Cytoplasmic determinants in *C. elegans* are segregated by cytoplasmic streaming and protein degradation: Par-protein and Mex-5/6 dependent cytoplasmic flow leads to posterior enrichment of P-granules (purple) and probably other determinants as well. Par-3/6 dependent posterior localization of Par1 restricts Mex-5/6 (green) into the anterior cytoplasm. Mex-5/6 inhibit localization of P-granules and Pie-1/Pos-1/Mex-1 (yellow) therein, possibly by protein degradation. Mex-5/6 subsequently clear residual determinants in the anterior daughter cell after cell division by ubiquitin-mediated protein degradation. (B) In *Drosophila* neuroblasts, apical Par-3/6 locally inactivate uniformly localized Lgl protein by aPKC-dependent phosphorylation. Lgl restricts active Myosin II to the apical cell cortex, where it prevents cortical determinant localization. Myosin VI (purple) promotes asymmetric localization of segregating determinants by vesicular transport.

[60,61]. As the human Pins homolog LGN was shown to bind NuMA [62], which in turn interacts with dynein [63], it is possible that G-proteins participate in the regulation of this important motor complex. Alternatively, G-proteins could act on microtubules directly, as mammalian Gαi binds tubulin and changes its polymerization behavior [64,65]. Most likely, multiple interactions between cortical polarity cues and the mitotic spindle are responsible for displacing and orienting mitotic spindles during asymmetric cell division.

Localizing Cell Fate Determinants

The hallmark of any intrinsically asymmetric cell division is the segregation of cell fate determining proteins or transcripts into one of the two daughter cells. This is achieved by polarizing the subcellular localization of these determinants during mitosis. Although the asymmetric localization of determinants is directed by the Par-3/6 complex both in *C. elegans* and *Drosophila*, the segregating determinants themselves are not conserved between the two systems. In *Drosophila*, cell fate determinants are generally localized at the cell cortex, while in *C. elegans*, they are localized in the cytoplasm. Consistent with this, distinct localization machineries seem to be operating in the two systems (Figure 5).

In *C. elegans* (Figure 5A), the CCCH-type Zinc-finger domain proteins Mex-1, Mex-5, Mex-6, Pie-1 and Pos-1 as well as cytoplasmic ribonucleoprotein complexes, called P-granules, segregate into one of the two daughter cells of the zygote. Asymmetric localization seems to involve two distinct steps. First, Mex-5/6 — two closely related, redundant proteins — are excluded from the posterior cytoplasm in a step that requires the kinase Par-1 [66]. Second, Par-proteins and Mex-5/6 induce a posterior flow of the central cytoplasm [18] to direct P-granules and probably also Mex-1, Pie-1 and Pos-1 into the posterior half of the cell. In addition, Mex-5/6 activate the DYRK family kinase Mbk-2 which contributes to the exclusion of Mex-1, Pie-1, Pos-1 and P-granules in the anterior cytoplasm [67,68]. This might involve local degradation of these proteins, as after division, residual amounts of Mex-1, Pie-1 and Pos-1 are cleared from the anterior daughter cell by Mex-5/6 and Mbk-2 dependent, ubiquitin mediated protein degradation [67,69]. Thus, cytoplasmic streaming and local degradation are responsible for determinant localization in *C. elegans*. As the *C. elegans* determinants are not conserved, it is unclear to what extent these results can be extrapolated to other organisms.

In *Drosophila* neuroblasts (Figure 5B), the apical Par-3/6 complex directs cell fate determinants to the opposite, basal cell cortex. Although *insc*, *pins* or *Gβ13F* mutants have defects in determinant localization, these phenotypes are less penetrant than in *par-3/6* mutants and might be indirect consequences of partial Par-3/6 mislocalization. The key target of the Par-3/6 is a cytoskeletal protein called Lethal(2) giant larvae (Lgl) [70]. In *lgl* mutants, apical localization of Par-3/6 is normal, but cell fate determinants fail to localize to the basal side and are partially lost from the cell cortex [70–72]. As in neuroblasts, determinants are localized opposite to Par-3/6 in SOP-cells and their asymmetric distribution is dependent on Par-3/6 [35,73] and Lgl [71], suggesting that similar mechanisms specify cell fate in the *Drosophila* central and peripheral nervous system.

Lgl is a 127 kDa protein that associates with membranes and the cytoskeleton [74]. *Drosophila lgl* mutants die from overproliferation of larval tissues, hence the name, and the protein has therefore been

classified as a tumor suppressor gene [75]. Lgl is conserved from yeast to humans and has a conserved function in cell polarity [13]. In neuroblasts, the protein is present both in the cytoplasm and around the cell cortex. It can bind directly to Par-6 and is an *in vivo* substrate for aPKC, the kinase present in the Par-3/6 complex [70]. Phosphorylation inactivates the Lgl protein and prevents its association with the actin cytoskeleton and with membranes. This has led to the hypothesis that the Par-3/6 complex phosphorylates Lgl at the apical cell cortex, whereas on the basal side the protein is in its active, unphosphorylated state and permits the recruitment of cell fate determinants [70].

How does Lgl recruit proteins to the cell cortex? Several results indicate that Lgl acts during asymmetric cell division by repressing non-muscle myosin II: Lgl can bind to the myosin II heavy chain and this interaction is suppressed by phosphorylation [76,77]. Indeed, mutations in myosin II suppress the *lgl* mutant phenotype [71,72,78]. Suppression of myosin II activity by a chemical inhibitor of Rho-kinase (ROCK) results in the same phenotype as overactivation of Lgl by expression of a form that can no longer be phosphorylated [79]. Finally, the amount of myosin II detected at the cell cortex is increased in *lgl* mutants [79]. Thus, Lgl promotes and myosin II inhibits localization of cell fate determinants to the cell cortex. How Lgl regulates myosin II and how myosin removes determinants from the cortex is unclear. In *C. elegans*, Par-proteins induce a myosin II dependent cortical flow [18]. Although an involvement of Lgl homologs has not been shown, asymmetric myosin activity could create a similar directional movement of the cytoplasm in *Drosophila* neuroblasts and thereby promote the asymmetric localization of determinants.

Besides its role in regulating myosin, Lgl seems to have a second function in the docking of vesicles to the plasma membrane. In yeast cells double mutant for the Lgl homologs SRO7 and SRO77, post-Golgi vesicles fail to fuse with the plasma membrane [80]. *Drosophila* Lgl can rescue this defect indicating that it might have a similar function [81]. Furthermore, vertebrate Lgl-homologs have been shown to bind to components of the vesicular transport machinery [82–84]. Although none of the segregating determinants have been shown to be in vesicles, a role for vesicle transport is supported by another mutant that acts downstream of Par-3/6 in neuroblasts: In neuroblasts mutant for the type VI myosin Jaguar, apical protein localization is normal but determinants fail to localize asymmetrically [85]. Jaguar can directly bind to the basal determinant Miranda and vesicle transport is the major function described for type VI myosins [85,86]. Therefore, Jaguar could transport vesicles carrying determinants and Lgl could allow their docking to the basal cell cortex.

In summary, asymmetric segregation of cell fate determinants is regulated by Par-proteins both in worms and flies. In *C. elegans*, Par-proteins induce a cytoplasmic flow and spatially restrict protein degradation to distribute determinants asymmetrically in the cytoplasm. In *Drosophila*, Par-3 and Par-6 localize the kinase aPKC, which phosphorylates and inactivates Lgl. Whether myosin dependent streaming or targeted vesicle docking (or any other unknown mechanisms) are responsible for determinant localization in flies remains to be seen.

Establishing Cell Fates

After asymmetric cell division, different amounts of determinants are present in the two daughter cells. Several mechanisms are known to translate these concentration differences into different cell fates. In *C. elegans*, the first cell divisions generate six founder blastomeres. Each of these blastomeres gives rise to clones with distinct cell fates [87]. Although the combinatorial action of segregating determinants makes it difficult to attribute cell fate decisions to individual proteins, transcriptional repression and translational regulation have emerged as principles of cell fate regulation. Examples are the repression of zygotic transcription in the germline by Pie-1 and the translational regulation of the Notch-like receptor Glp-1 by Pos-1 [88–90].

In *Drosophila*, different sets of determinants are responsible for cell fate decisions in neuroblasts and SOP-cells. In neuroblasts, the coiled coil protein Miranda segregates into the basal ganglion mother cell [91,92]. Miranda acts as an adaptor that binds and transports two important determinants, Prospero [93,94] and Staufen [95,96]. Prospero is a homeodomain containing transcription factor that enters the nucleus of the ganglion mother cell where it induces and represses various cell type specific target genes. Staufen is a RNA binding protein that binds *prospero* transcripts and localises them into the ganglion mother cell as well. Although localization of *prospero* RNA is not essential, it serves as an important backup mechanism [95]. Thus, cell fate decisions in neuroblasts are essentially made by differential transcriptional regulation.

In SOP-cells, the key determinants are the phosphotyrosine binding domain protein Numb [3] and the E3 ubiquitin ligase Neuralized [97] (Figure 6A). Both proteins regulate signal transduction through the transmembrane receptor Notch by controlling the endocytosis of key components of the Notch/Delta system (Figure 6B). Although Notch and its ligand Delta are present in both daughter cells of the SOP, their signaling activity is biased by Numb and Neuralized. Numb binds to α-Adaptin, a component of the AP-2 complex, which in turn binds to transmembrane proteins and targets them for endocytosis [98,99]. Numb polarizes the distribution of α -Adaptin so that higher amounts segregate into the pIIb cell [98]. Because Numb can also bind to the Notch receptor [100], this has led to the hypothesis that Numb represses Notch in the pIIb cell by targeting the receptor for endocytosis (Figure 6C). Numb also binds to Sanpodo, a four-pass transmembrane protein that binds to Notch and is required for Notch signaling in neuroblasts [101] (Figure 6C). Whether Notch itself or Sanpodo is the key target of Numb mediated endocytosis in SOP-cells remains to be determined.

Neuralized influences Notch signaling by ubiquitinating its ligand Delta [97,102,103] (Figure 6B). As

Neuralized is required both for the internalization of Delta and for the activation of Notch [97,104,105], this has led to a model according to which Neuralized ubiquitinates Delta in the pIIb cell which in turn activates Notch in the pIIa cell [97]. Thus, Numb represses Notch in the pIIb cell while Neuralized activates Notch in the pIIa cell, which generates a strong bias of cell–cell signaling between both daughter cells in the SOP-cell lineage.

Asymmetric Cell Division in Vertebrates

Many of the proteins involved in invertebrate asymmetric cell divisions are conserved in mammals, the G-proteins, Pins, the Par-proteins, Lgl and Numb. Thus, they might be involved in the generation of cell fate diversity during vertebrate development. Consistent with this, intrinsically asymmetric cell divisions that produce daughter cells with different fates have been described in the progenitor cells of neurons and glia in the vertebrate brain [106–109]. Similar to invertebrates, the orientation of cell divisions seems to correlate with cell fates. In mouse and rat neuroepithelial cells, divisions in the plane of the epithelium generate symmetric daughter cells, whereas apical–basal cell divisions generate asymmetric ones. While this reorientation of cell divisions along the apical-basal axis is similar to *Drosophila* neuroblast divisions, zebrafish retinal progenitors apparently reorient their mitotic spindle according to a planar polarity axis [110] and thus resemble *Drosophila* SOP-cell divisions. Therefore, it seems that vertebrate neural progenitor cell divisions exhibit characteristic features of invertebrate asymmetric cell divisions, suggesting the involvement of segregating determinants.

Indeed, vertebrate Numb localizes asymmetrically in mouse, rat and chicken neural progenitor cells and asymmetric segregation of Numb has been shown to correlate with asymmetric cell fate in clonal density cultures of mouse cortical progenitor cells [111–114]. However, a second Numb-homolog, called Numblike, is uniformly distributed in the cytoplasm [115]. Surprisingly, both proteins act redundantly and perform two different roles in the developing mouse brain [116–118]: During early neural development, Numb and Numblike regulate the proliferation of neural progenitors. In *numb/numblike* double knockouts, the progenitor pool is depleted and an accompanying transient wave of neuron overproduction suggests that this is due to all their descendants differentiating into neurons [116]. During later stages of neurogenesis, Numb and Numblike are also required for neuronal differentiation of progenitor cells, consistent with the generation of neurons by asymmetric cell divisions during this phase [117]. As in *Drosophila*, Numb segregates into one of the two daughter cells and is required for correct specification of its fate. However, vertebrate Numb-proteins have a dual role during neurogenesis, which might rely on different splice variants [119,120] or on different prognitor cell subtypes: Numb and Numblike are required to both inhibit [116,118] and promote [117] neuronal differentiation.

The parallels between *Drosophila* and vertebrate asymmetric cell divisions seem to extend to factors

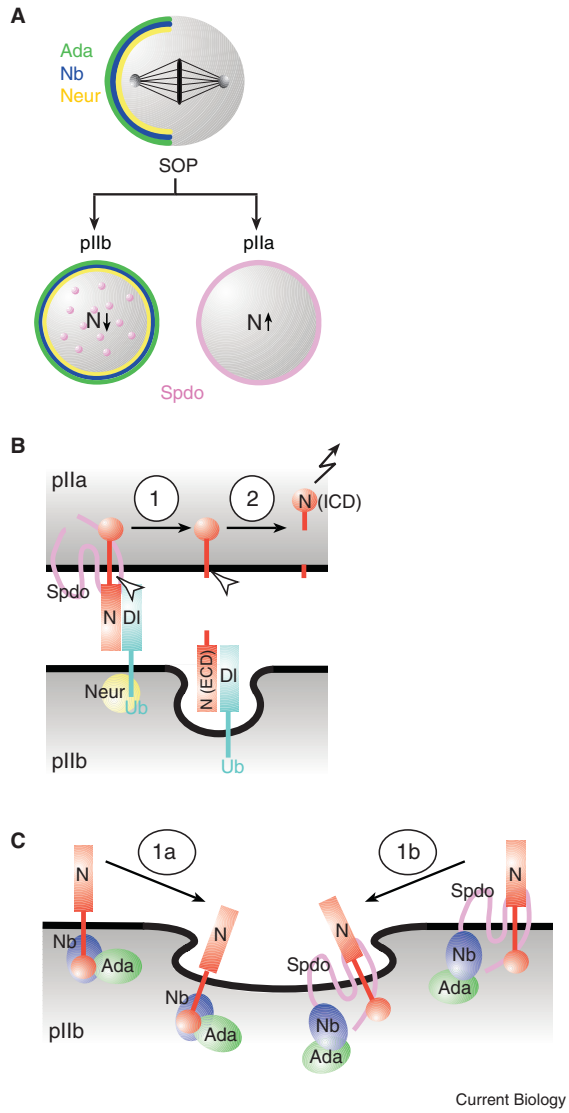

Figure 6. Cell fate specification after SOP-cell division.
(A) During SOP-cell division, the determinants Numb (Nb), α-Adaptin (Ada) and Neuralized (Neur) segregate asymmetrically into the pIIb cell, where they inhibit Notch signaling. (B) The E3 ubiquitin ligase Neuralized ubiquitinates Delta (Dl) in the pIIb cell (1) and facilitates cleavage (arrowhead) of Notch (N) in the pIIa cell. Upon cleavage, the extracellular domain (ECD) of Notch enters the pIIb cell by transcytosis. The remaining fragment is cleaved again (arrowhead) and the intracellular domain of Notch (ICD) is released and enters the nucleus (2). (C) Notch inhibition in the pIIb cell. Numb and its binding partner α-Adaptin both bind to Notch and Sanpodo (Spdo). Sanpodo is required for Notch signaling, and either endocytosis of Notch (1a) or Sanpodo (1b) is responsible for Notch inhibition.

acting upstream and downstream of Numb: In dividing neural progenitor cells of the mouse brain that lack the *Drosophila* Lgl homolog, Lgl1, Numb segregates into both daughter cells [121] and consequently *lgl1* mutant progenitors fail to differentiate. Thus, elements of the *Drosophila* Numb localization machinery seem to be conserved. Furthermore, vertebrate Numb can bind to Notch and inhibit its activity [113,122,123]. Like loss of Numb and Numblike, Notch overexpression or

ectopic Notch activation inhibits neuronal differentiation [113,117,124,125], suggesting that elements of the Numb downstream machinery are also conserved between *Drosophila* and vertebrates.

Despite these similarities, however, there are characteristic differences. First, mammalian Numb is apically enriched in asymmetrically dividing progenitor cells, while in *Drosophila* the protein is segregated into the basal daughter cell. As apical localization of Par-proteins is conserved [126,127], this would suggest a Par-3/6 independent, or at least different, mechanism for Numb localization in vertebrates. Second, the orientation of most precursor divisions in the mouse brain is not along the apical-basal axis so that Numb is not inherited by only one daughter cell [111]. Because the number of apical–basal divisions, however, does not account for the amount of neuronal differentiation [128], many parallel divisions must produce different daughter cells, indicating that Numb-independent mechanisms for cell fate determination do exist. Asymmetric inheritance of morphological processes [129] and apical membrane domains [126] could contribute to differential cell fate specification after progenitor divisions, but up to now functional evidence for these possibilities is missing.

Besides neural development, mouse homologs of Numb also act in muscle progenitors where they are thought to repress Notch [130,131]. The recent discovery of asymmetric Par-6 localization in mouse oocytes [132] suggests that the analysis of asymmetric cell division in vertebrates might still deliver some surprises.

Perspectives

In the past years, a conserved general mechanism for asymmetric cell division has been discovered: asymmetric localization of Par-proteins polarizes the cell cortex, orients the mitotic spindle through heterotrimeric G-proteins and directs the segregation of determinants into only one of the two daughter cells.

Despite this progress, some of the most burning questions are still open: How are determinants transported into one of the two daughter cells? How are Par-proteins anchored at the cell cortex and how is their asymmetric distribution achieved? How do G-proteins act on microtubules and how is this process regulated by the Par-proteins?

The remarkable conservation of Numb and other proteins has suggested that asymmetric cell division is a fundamental process for the development of many different systems, not the least our own brain. In parallel, new and remarkable functions for molecules involved in asymmetric cell division are identified in other aspects of cellular polarity [133]. Although *Drosophila* and *C. elegans* have been very successful in the past, many answers to unresolved questions in the future might come from vertebrate models and other cellular systems.

Acknowledgments

We would like to thank all members of the Knoblich lab for inspiring discussions and Michel Cayouette and Frank Schnorrer for helpful comments on the manuscript. Work in the Knoblich lab is supported by Boehringer Ingelheim, the Austrian Science Fund (FWF) and the Austrian Academy of Sciences.

References

1. Horvitz, H.R., and Herskowitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell *68*, 237–255.
2. Conklin, E.G. (1905). The organization and cell-lineage of the ascidian egg. J. Acad. Nat. Sci. Philadelphia *13*, 1–119.
3. Rhyu, M.S., Jan, L.Y., and Jan, Y.N. (1994). Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell *76*, 477–491.
4. Knoblich, J.A. (2001). Asymmetric cell division during animal development. Nat. Rev. Mol. Cell. Biol. *2*, 11–20.
5. Doe, C.Q., and Bowerman, B. (2001). Asymmetric cell division: fly neuroblast meets worm zygote. Curr. Opin. Cell Biol. *13*, 68–75.
6. Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.
7. Lyczak, R., Gomes, J.E., and Bowerman, B. (2002). Heads or tails: cell polarity and axis formation in the early *Caenorhabditis elegans* embryo. Dev. Cell *3*, 157–166.
8. Schneider, S.Q., and Bowerman, B. (2003). Cell polarity and the cytoskeleton in the *Caenorhabditis elegans* zygote. Annu. Rev. Genet. *37*, 221–249.
9. Jan, Y.N., and Jan, L.Y. (2001). Asymmetric cell division in the *Drosophila* nervous system. Nat. Rev. Neurosci. *2*, 772–779.
10. Bardin, A.J., Le Borgne, R., and Schweisguth, F. (2004). Asymmetric localization and function of cell-fate determinants: a fly's view. Curr. Opin. Neurobiol. *14*, 6–14.
11. Kemphues, K.J., Priess, J.R., Morton, D.G., and Cheng, N.S. (1988). Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. Cell *52*, 311–320.
12. Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K.J., Miwa, J., and Ohno, S. (1998). Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. Development *125*, 3607–3614.
13. Macara, I.G. (2004). Parsing the polarity code. Nat. Rev. Mol. Cell. Biol. *5*, 220–231.
14. Ohno, S. (2001). Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. Curr. Opin. Cell Biol. *13*, 641–648.
15. Wallenfang, M.R., and Seydoux, G. (2000). Polarization of the anterior-posterior axis of *C. elegans* is a microtubule-directed process. Nature *408*, 89–92.
16. Cuenca, A.A., Schetter, A., Aceto, D., Kemphues, K., and Seydoux, G. (2003). Polarization of the *C. elegans* zygote proceeds via distinct establishment and maintenance phases. Development *130*, 1255–1265.
17. Cowan, C. and Hyman, A.A. (2004). Centrosomes direct cell polarity independently of their role in microtubule assembly in *C. elegans* embryos. Nature, *in press*.
18. Cheeks, R.J., Canman, J.C., Gabriel, W.N., Meyer, N., Strome, S., and Goldstein, B. (2004). *C. elegans* PAR proteins function by mobilizing and stabilizing asymmetrically localized protein complexes. Curr. Biol. *14*, 851–862.
19. Gotta, M., Abraham, M.C., and Ahringer, J. (2001). CDC-42 controls early cell polarity and spindle orientation in *C. elegans*. Curr. Biol. *11*, 482–488.
20. Petronczki, M., and Knoblich, J.A. (2001). DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in *Drosophila*. Nat. Cell Biol. *3*, 43–49.
21. Schober, M., Schaefer, M., and Knoblich, J.A. (1999). Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. Nature *402*, 548–551.
22. Wodarz, A., Ramrath, A., Kuchinke, U., and Knust, E. (1999). Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. Nature *402*, 544–547.
23. Kuchinke, U., Grawe, F., and Knust, E. (1998). Control of spindle orientation in *Drosophila* by the Par-3-related PDZ-domain protein Bazooka. Curr. Biol. *8*, 1357–1365.
24. Wodarz, A., Ramrath, A., Grimm, A., and Knust, E. (2000). *Drosophila* atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. J. Cell Biol. *150*, 1361–1374.
25. Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., and Knoblich, J.A. (1996). Role of inscuteable in orienting asymmetric cell divisions in *Drosophila*. Nature *383*, 50–55.

26. Yu, F., Morin, X., Cai, Y., Yang, X., and Chia, W. (2000). Analysis of partner of inscuteable, a novel player of *Drosophila* asymmetric divisions, reveals two distinct steps in inscuteable apical localization. Cell *100*, 399–409.

27. Schaefer, M., Shevchenko, A., and Knoblich, J.A. (2000). A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in *Drosophila*. Curr. Biol. *10*, 353–362.

28. Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A., Bryant, P., and O'Kane, C.J. (2000). Rapsynoid/Partner of Inscuteable controls asymmetric division of larval neuroblasts in *Drosophila*. J. Neurosci. (Online) *20*, RC84.

29. Schaefer, M., Petronczki, M., Dorner, D., Forte, M., and Knoblich, J.A. (2001). Heterotrimeric G-proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system. Cell *107*, 183–194.

30. Cai, Y., Yu, F., Lin, S., Chia, W., and Yang, X. (2003). Apical complex genes control mitotic spindle geometry and relative size of daughter cells in *Drosophila* neuroblast and pI asymmetric divisions. Cell *112*, 51–62.

31. Broadus, J., and Doe, C.Q. (1997). Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in *Drosophila* neuroblasts. Curr. Biol. *7*, 827–835.

32. Butty, A.C., Perrinjaquet, N., Petit, A., Jaquenoud, M., Segall, J.E., Hofmann, K., Zwahlen, C., and Peter, M. (2002). A positive feedback loop stabilizes the guanine-nucleotide exchange factor Cdc24 at sites of polarization. EMBO J. *21*, 1565–1576.

33. Lu, B., Usui, T., Uemura, T., Jan, L., and Jan, Y.N. (1999). Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in *Drosophila*. Curr. Biol. *9*, 1247–1250.

34. Roegiers, F., Younger-Shepherd, S., Jan, L.Y., and Jan, Y.N. (2001). Two types of asymmetric divisions in the *Drosophila* sensory organ precursor cell lineage. Nat. Cell Biol. *3*, 58–67.

35. Bellaiche, Y., Radovic, A., Woods, D.F., Hough, C.D., Parmentier, M., O'Kane, C.J., Bryant, P.J., and Schweisguth, F. (2001). The partner of inscuteable/discs-large complex is required to establish planar polarity during asymmetric cell division in drosophila. Cell *106*, 355–366.

36. Bellaiche, Y., Beaudoin-Massiani, O., Stuttem, I., and Schweisguth, F. (2004). The planar cell polarity protein Strabismus promotes Pins anterior localization during asymmetric division of sensory organ precursor cells in *Drosophila*. Development *131*, 469–478.

37. Adler, P.N. (2002). Planar signaling and morphogenesis in *Drosophila*. Dev. Cell *2*, 525–535.

38. Hyman, A.A., and White, J.G. (1987). Determination of cell division axes in the early embryogenesis of *Caenorhabditis elegans*. J. Cell Biol. *105*, 2123–2135.

39. Gonczy, P. (2002). Mechanisms of spindle positioning: focus on flies and worms. Trends Cell Biol*. 12*, 332–339.

40. Hyman, A.A. (1989). Centrosome movement in the early divisions of *Caenorhabditis elegans*: a cortical site determining centrosome position. J. Cell Biol. *109*, 1185–1193.

41. Grill, S.W., Gonczy, P., Stelzer, E.H., and Hyman, A.A. (2001). Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. Nature *409*, 630–633.

42. Kaltschmidt, J.A., Davidson, C.M., Brown, N.H., and Brand, A.H. (2000). Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. Nat. Cell Biol. *2*, 7–12.

43. Zwaal, R.R., Ahringer, J., van Luenen, H.G., Rushforth, A., Anderson, P., and Plasterk, R.H. (1996). G proteins are required for spatial orientation of early cell cleavages in *C. elegans* embryos. Cell *86*, 619–629.

44. Tsou, M.F., Hayashi, A., and Rose, L.S. (2003). LET-99 opposes G{alpha}/GPR signaling to generate asymmetry for spindle positioning in response to PAR and MES-1/SRC-1 signaling. Development *130*, 5717-5730.

45. Gotta, M., and Ahringer, J. (2001). Distinct roles for Galpha and Gbetagamma in regulating spindle position and orientation in *Caenorhabditis elegans* embryos. Nat. Cell Biol. *3*, 297–300.

46. Izumi, Y., Ohta, N., Itoh-Furuya, A., Fuse, N., and Matsuzaki, F. (2004). Differential functions of G protein and Baz-aPKC signaling pathways in *Drosophila* neuroblast asymmetric division. J. Cell Biol. *164*, 729-738.

47. Yu, F., Cai, Y., Kaushik, R., Yang, X., and Chia, W. (2003). Distinct roles of Galphai and Gbeta13F subunits of the heterotrimeric G protein complex in the mediation of *Drosophila* neuroblast asymmetric divisions. J. Cell Biol. *162*, 623–633.

48. Gotta, M., Dong, Y., Peterson, Y.K., Lanier, S.M., and Ahringer, J. (2003). Asymmetrically distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo. Curr. Biol. *13*, 1029–1037.

49. Hamm, H.E. (1998). The many faces of G protein signaling. J. Biol. Chem. *273*, 669–672.

50. Siderovski, D.P., Diverse-Pierluissi, M., and De Vries, L. (1999). The GoLoco motif: a Galphai/o binding motif and potential guanine-nucleotide exchange factor. Trends Biochem. Sci. *24*, 340–341.

51. Kimple, R.J., Willard, F.S., and Siderovski, D.P. (2002). The GoLoco motif: heralding a new tango between G protein signaling and cell division. Mol. Intervent. *2*, 88–100.

52. Gonczy, P., Echeverri, C., Oegema, K., Coulson, A., Jones, S.J., Copley, R.R., Duperon, J., Oegema, J., Brehm, M., Cassin, E., Hannak, E., Kirkham, M., Pichler, S., Flohrs, K., Goessen, A., Leidel, S., Alleaume, A.M., Martin, C., Ozlu, N., Bork, P., and Hyman, A.A. (2000). Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. Nature *408*, 331–336.

53. Colombo, K., Grill, S.W., Kimple, R.J., Willard, F.S., Siderovski, D.P., and Gonczy, P. (2003). Translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos. Science *300*, 1957–1961.

54. Gotta, M., Dong, Y., Peterson, Y.K., Lanier, S.M., and Ahringer, J. (2003). Asymmetrically distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo. Curr. Biol. *13*, 1029–1037.

55. Srinivasan, D.G., Fisk, R.M., Xu, H., and Van Den Heuvel, S. (2003). A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in *C. elegans*. Genes Dev. *17*, 1225–1239.

56. Tsou, M.F., Hayashi, A., DeBella, L.R., McGrath, G., and Rose, L.S. (2002). LET-99 determines spindle position and is asymmetrically enriched in response to PAR polarity cues in *C. elegans* embryos. Development *129*, 4469–4481.

57. Fuse, N., Hisata, K., Katzen, A.L., and Matsuzaki, F. (2003). Heterotrimeric g proteins regulate daughter cell size asymmetry in *Drosophila* neuroblast divisions. Curr. Biol. *13*, 947–954.

58. Nishimura, T., Kato, K., Yamaguchi, T., Fukata, Y., Ohno, S., and Kaibuchi, K. (2004). Role of the PAR-3–KIF3 complex in the establishment of neuronal polarity. Nat. Cell Biol. *6*, 328–334.

59. Grill, S.W., Howard, J., Schaffer, E., Stelzer, E.H., and Hyman, A.A. (2003). The distribution of active force generators controls mitotic spindle position. Science *301*, 518–521.

60. Skop, A.R., and White, J.G. (1998). The dynactin complex is required for cleavage plane specification in early *Caenorhabditis elegans* embryos. Curr. Biol. *8*, 1110–1116.

61. Gonczy, P., Pichler, S., Kirkham, M., and Hyman, A.A. (1999). Cytoplasmic dynein is required for distinct aspects of MTOC positioning, including centrosome separation, in the one cell stage *Caenorhabditis elegans* embryo. J. Cell Biol. *147*, 135–150.

62. Du, Q., Stukenberg, P.T., and Macara, I.G. (2001). A mammalian Partner of inscuteable binds NuMA and regulates mitotic spindle organization. Nat. Cell Biol. *3*, 1069–1075.

63. Merdes, A., Ramyar, K., Vechio, J.D., and Cleveland, D.W. (1996). A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. Cell *87*, 447–458.

64. Wang, N., Yan, K., and Rasenick, M.M. (1990). Tubulin binds specifically to the signal-transducing proteins, Gs alpha and Gi alpha 1. J. Biol. Chem. *265*, 1239–1242.

65. Roychowdhury, S., Panda, D., Wilson, L., and Rasenick, M.M. (1999). G protein alpha subunits activate tubulin GTPase and modulate microtubule polymerization dynamics. J. Biol. Chem. *274*, 13485–13490.

66. Schubert, C.M., Lin, R., de Vries, C.J., Plasterk, R.H., and Priess, J.R. (2000). MEX-5 and MEX-6 function to establish soma/germline asymmetry in early *C. elegans* embryos. Mol. Cell *5*, 671–682.

67. Pellettieri, J., Reinke, V., Kim, S.K., and Seydoux, G. (2003). Coordinate activation of maternal protein degradation during the egg-to-embryo transition in *C. elegans*. Dev. Cell *5*, 451–462.

68. Pang, K.M., Ishidate, T., Nakamura, K., Shirayama, M., Trzepacz, C., Schubert, C.M., Priess, J.R., and Mello, C.C. (2004). The minibrain kinase homolog, mbk-2, is required for spindle positioning and asymmetric cell division in early *C. elegans* embryos. Dev. Biol. *265*, 127–139.

69. DeRenzo, C., Reese, K.J., and Seydoux, G. (2003). Exclusion of germ plasm proteins from somatic lineages by cullin-dependent degradation. Nature *424*, 685–689.

70. Betschinger, J., Mechtler, K., and Knoblich, J.A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature *422*, 326–330.

71. Ohshiro, T., Yagami, T., Zhang, C., and Matsuzaki, F. (2000). Role of cortical tumour-suppressor proteins in asymmetric division of *Drosophila* neuroblast. Nature *408*, 593–596.

72. Peng, C.Y., Manning, L., Albertson, R., and Doe, C.Q. (2000). The tumour-suppressor genes lgl and dlg regulate basal protein targeting in *Drosophila* neuroblasts. Nature *408*, 596–600.

73. Roegiers, F., Younger-Shepherd, S., Jan, L.Y., and Jan, Y.N. (2001). Bazooka is required for localization of determinants and controlling proliferation in the sensory organ precursor cell lineage in *Drosophila*. Proc. Natl. Acad. Sci. USA *98*, 14469–14474.

74. Strand, D., Raska, I., and Mechler, B.M. (1994). The *Drosophila* lethal(2)giant larvae tumor suppressor protein is a component of the cytoskeleton. J. Cell Biol. *127*, 1345–1360.

75. De Lorenzo, C., Mechler, B.M., and Bryant, P.J. (1999). What is *Drosophila* telling us about cancer? Cancer Metastasis Rev. *18*, 295–311.

76. Strand, D., Jakobs, R., Merdes, G., Neumann, B., Kalmes, A., Heid, H.W., Husmann, I., and Mechler, B.M. (1994). The *Drosophila* lethal(2)giant larvae tumor suppressor protein forms homo-oligomers and is associated with nonmuscle myosin II heavy chain. J. Cell Biol. *127*, 1361–1373.

77. Kalmes, A., Merdes, G., Neumann, B., Strand, D., and Mechler, B.M. (1996). A serine-kinase associated with the p127-l(2)gl tumour suppressor of *Drosophila* may regulate the binding of p127 to nonmuscle myosin II heavy chain and the attachment of p127 to the plasma membrane. J. Cell Sci. *109*, 1359–1368.

78. Albertson, R., and Doe, C.Q. (2003). Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. Nat. Cell Biol. *5*, 166–170.

79. Barros, C.S., Phelps, C.B., and Brand, A.H. (2003). *Drosophila* nonmuscle myosin II promotes the asymmetric segregation of cell fate determinants by cortical exclusion rather than active transport. Dev. Cell *5*, 829–840.

80. Lehman, K., Rossi, G., Adamo, J.E., and Brennwald, P. (1999). Yeast homologues of tomosyn and lethal giant larvae function in exocytosis and are associated with the plasma membrane SNARE, Sec9. J. Cell Biol. *146*, 125–140.

81. Larsson, K., Bohl, F., Sjostrom, I., Akhtar, N., Strand, D., Mechler, B.M., Grabowski, R., and Adler, L. (1998). The Saccharomyces cerevisiae SOP1 and SOP2 genes, which act in cation homeostasis, can be functionally substituted by the *Drosophila* lethal(2)giant larvae tumor suppressor gene. J. Biol. Chem. *273*, 33610–33618.

82. Musch, A., Cohen, D., Yeaman, C., Nelson, W.J., Rodriguez-Boulan, E., and Brennwald, P.J. (2002). Mammalian homolog of *Drosophila* tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. Mol. Biol. Cell *13*, 158–168.

83. Fujita, Y., Shirataki, H., Sakisaka, T., Asakura, T., Ohya, T., Kotani, H., Yokoyama, S., Nishioka, H., Matsuura, Y., Mizoguchi, A., Scheller, R.H., and Takai, Y. (1998). Tomosyn: a syntaxin-1-binding protein that forms a novel complex in the neurotransmitter release process. Neuron *20*, 905–915.

84. Masuda, E.S., Huang, B.C., Fisher, J.M., Luo, Y., and Scheller, R.H. (1998). Tomosyn binds t-SNARE proteins via a VAMP-like coiled coil. Neuron *21*, 479–480.

85. Petritsch, C., Tavosanis, G., Turck, C.W., Jan, L.Y., and Jan, Y.N. (2003). The *Drosophila* myosin VI Jaguar is required for basal protein targeting and correct spindle orientation in mitotic neuroblasts. Dev. Cell *4*, 273–281.

86. Buss, F., Luzio, J.P., and Kendrick-Jones, J. (2002). Myosin VI, an actin motor for membrane traffic and cell migration. Traffic *3*, 851–858.

87. Rose, L.S., and Kemphues, K.J. (1998). Early patterning of the *C. elegans* embryo. Annu. Rev. Genet. *32*, 521–545.

88. Seydoux, G., Mello, C.C., Pettitt, J., Wood, W.B., Priess, J.R., and Fire, A. (1996). Repression of gene expression in the embryonic germ lineage of *C. elegans*. Nature *382*, 713–716.

89. Evans, T.C., Crittenden, S.L., Kodoyianni, V., and Kimble, J. (1994). Translational control of maternal glp-1 mRNA establishes an asymmetry in the *C. elegans* embryo. Cell *77*, 183–194.

90. Ogura, K., Kishimoto, N., Mitani, S., Gengyo-Ando, K., and Kohara, Y. (2003). Translational control of maternal glp-1 mRNA by POS-1 and its interacting protein SPN-4 in *Caenorhabditis elegans*. Development *130*, 2495–2503.

91. Shen, C.P., Jan, L.Y., and Jan, Y.N. (1997). Miranda is required for the asymmetric localization of Prospero during mitosis in *Drosophila*. Cell *90*, 449–458.

92. Ikeshima-Kataoka, H., Skeath, J.B., Nabeshima, Y., Doe, C.Q., and Matsuzaki, F. (1997). Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. Nature *390*, 625–629.

93. Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L.Y., and Jan, Y.N. (1991). prospero is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. Cell *67*, 941–953.

94. Doe, C.Q., Chu-LaGraff, Q., Wright, D.M., and Scott, M.P. (1991). The prospero gene specifies cell fates in the *Drosophila* central nervous system. Cell *65*, 451–464.

95. Broadus, J., Fuerstenberg, S., and Doe, C.Q. (1998). Staufen-dependent localization of prospero mRNA contributes to neuroblast daughter-cell fate. Nature *391*, 792–795.

96. Shen, C.P., Knoblich, J.A., Chan, Y.M., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1998). Miranda as a multidomain adapter linking apically localized Inscuteable and basally localized Staufen and Prospero during asymmetric cell division in *Drosophila*. Genes Dev. *12*, 1837–1846.

97. Le Borgne, R., and Schweisguth, F. (2003). Unequal segregation of Neuralized biases Notch activation during asymmetric cell division. Dev. Cell *5*, 139–148.

98. Berdnik, D., Torok, T., Gonzalez-Gaitan, M., and Knoblich, J.A. (2002). The endocytic protein alpha-Adaptin is required for numb-mediated asymmetric cell division in *Drosophila*. Dev. Cell *3*, 221–231.

99. Takei, K., and Haucke, V. (2001). Clathrin-mediated endocytosis: membrane factors pull the trigger. Trends Cell Biol. *11*, 385–391.

100. Guo, M., Jan, L.Y., and Jan, Y.N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. Neuron *17*, 27–41.

101. O'Connor-Giles, K.M., and Skeath, J.B. (2003). Numb inhibits membrane localization of sanpodo, a four-pass transmembrane protein, to promote asymmetric divisions in *Drosophila*. Dev. Cell *5*, 231–243.

102. Lai, E.C., Deblandre, G.A., Kintner, C., and Rubin, G.M. (2001). *Drosophila* neuralized is a ubiquitin ligase that promotes the internalization and degradation of delta. Dev. Cell *1*, 783–794.

103. Pavlopoulos, E., Pitsouli, C., Klueg, K.M., Muskavitch, M.A., Moschonas, N.K., and Delidakis, C. (2001). neuralized Encodes a peripheral membrane protein involved in delta signaling and endocytosis. Dev. Cell *1*, 807–816.

104. Parks, A.L., Klueg, K.M., Stout, J.R., and Muskavitch, M.A. (2000). Ligand endocytosis drives receptor dissociation and activation in the Notch pathway. Development *127*, 1373–1385.

105. Li, Y., and Baker, N.E. (2004). The roles of cis-inactivation by Notch ligands and of neuralized during eye and bristle patterning in *Drosophila*. BMC Dev. Biol. *4*, 5.

106. Chenn, A., and McConnell, S.K. (1995). Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. Cell *82*, 631–641.

107. Cayouette, M., and Raff, M. (2003). The orientation of cell division influences cell-fate choice in the developing mammalian retina. Development *130*, 2329–2339.

108. Haydar, T.F., Ang, E., Jr., and Rakic, P. (2003). Mitotic spindle rotation and mode of cell division in the developing telencephalon. Proc. Natl. Acad. Sci. USA *100*, 2890–2895.

109. Noctor, S.C., Martinez-Cerdeno, V., Ivic, L., and Kriegstein, A.R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat. Neurosci. *7*, 136-144.

110. Das, T., Payer, B., Cayouette, M., and Harris, W.A. (2003). In vivo time-lapse imaging of cell divisions during neurogenesis in the developing zebrafish retina. Neuron *37*, 597–609.

111. Zhong, W., Feder, J.N., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1996). Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis. Neuron *17*, 43–53.

112. Cayouette, M., Whitmore, A.V., Jeffery, G., and Raff, M. (2001). Asymmetric segregation of Numb in retinal development and the influence of the pigmented epithelium. J. Neurosci. *21*, 5643–5651.

113. Wakamatsu, Y., Maynard, T.M., Jones, S.U., and Weston, J.A. (1999). NUMB localizes in the basal cortex of mitotic avian neuroepithelial cells and modulates neuronal differentiation by binding to NOTCH-1. Neuron *23*, 71–81.

114. Shen, Q., Zhong, W., Jan, Y.N., and Temple, S. (2002). Asymmetric Numb distribution is critical for asymmetric cell division of mouse cerebral cortical stem cells and neuroblasts. Development *129*, 4843–4853.

115. Zhong, W., Jiang, M.M., Weinmaster, G., Jan, L.Y., and Jan, Y.N. (1997). Differential expression of mammalian Numb, Numblike and Notch1 suggests distinct roles during mouse cortical neurogenesis. Development *124*, 1887–1897.

116. Petersen, P.H., Zou, K., Hwang, J.K., Jan, Y.N., and Zhong, W. (2002). Progenitor cell maintenance requires numb and numblike during mouse neurogenesis. Nature *419*, 929–934.

117. Li, H.S., Wang, D., Shen, Q., Schonemann, M.D., Gorski, J.A., Jones, K.R., Temple, S., Jan, L.Y., and Jan, Y.N. (2003). Inactivation of Numb and Numblike in embryonic dorsal forebrain impairs neurogenesis and disrupts cortical morphogenesis. Neuron *40*, 1105–1118.

118. Petersen, P.H., Zou, K., Krauss, S., and Zhong, W. (2004). Continuing role for mouse Numb and Numbl in maintaining progenitor cells during cortical neurogenesis. Nat. Neurosci. *7*, 803–811.

119. Dooley, C.M., James, J., Jane McGlade, C., and Ahmad, I. (2003). Involvement of numb in vertebrate retinal development: Evidence for multiple roles of numb in neural differentiation and maturation. J. Neurobiol. *54*, 313–325.

120. Verdi, J.M., Bashirullah, A., Goldhawk, D.E., Kubu, C.J., Jamali, M., Meakin, S.O., and Lipshitz, H.D. (1999). Distinct human NUMB isoforms regulate differentiation vs. proliferation in the neuronal lineage. Proc. Natl. Acad. Sci. USA *96*, 10472–10476.

121. Klezovitch, O., Fernandez, T.E., Tapscott, S.J., and Vasioukhin, V. (2004). Loss of cell polarity causes severe brain dysplasia in Lgl1 knockout mice. Genes Dev. *18*, 559–571.

122. Berezovska, O., McLean, P., Knowles, R., Frosh, M., Lu, F.M., Lux, S.E., and Hyman, B.T. (1999). Notch1 inhibits neurite outgrowth in postmitotic primary neurons. Neuroscience *93*, 433–439.

123. Sestan, N., Artavanis-Tsakonas, S., and Rakic, P. (1999). Contact-dependent inhibition of cortical neurite growth mediated by notch signaling. Science *286*, 741–746.

124. Lardelli, M., Williams, R., Mitsiadis, T., and Lendahl, U. (1996). Expression of the Notch 3 intracellular domain in mouse central nervous system progenitor cells is lethal and leads to disturbed neural tube development. Mech. Dev. *59*, 177–190.

125. Ohtsuka, T., Sakamoto, M., Guillemot, F., and Kageyama, R. (2001). Roles of the basic helix-loop-helix genes Hes1 and Hes5 in expansion of neural stem cells of the developing brain. J. Biol. Chem. *276*, 30467–30474.

126. Kosodo, Y., Roper, K., Haubensak, W., Marzesco, A.M., Corbeil, D., and Huttner, W.B. (2004). Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. EMBO J. *23*, 2314-2324.

127. Manabe, N., Hirai, S., Imai, F., Nakanishi, H., Takai, Y., and Ohno, S. (2002). Association of ASIP/mPAR-3 with adherens junctions of mouse neuroepithelial cells. Dev. Dyn. *225*, 61–69.

128. Huttner, W.B., and Brand, M. (1997). Asymmetric division and polarity of neuroepithelial cells. Curr. Opin. Neurobiol. *7*, 29–39.

129. Miyata, T., Kawaguchi, A., Okano, H., and Ogawa, M. (2001). Asymmetric inheritance of radial glial fibers by cortical neurons. Neuron *31*, 727–741.

130. Ruiz Gomez, M., and Bate, M. (1997). Segregation of myogenic lineages in *Drosophila* requires numb. Development *124*, 4857–4866.

131. Conboy, I.M., and Rando, T.A. (2002). The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Dev Cell *3*, 397–409.

132. Vinot, S., Le, T., Maro, B., and Louvet-Vallee, S. (2004). Two PAR6 proteins become asymmetrically localized during establishment of polarity in mouse oocytes. Curr. Biol. *14*, 520–525.

133. Shi, S.H., Jan, L.Y., and Jan, Y.N. (2003). Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. Cell *112*, 63–75.